

## THE PROMPT DETECTION OF IONIZING RADIATIONS BY CARPENTER ANTS<sup>1</sup>

DAVID L. MARTINSEN<sup>2</sup> AND DONALD J. KIMELDORF

*The Radiation Center, Oregon State University, Corvallis, Oregon 97331*

The ability of animals to detect ionizing radiations has been known almost since the discovery of x-rays, but only recently has it become widely appreciated. The prompt detection of ionizing radiation has now been thoroughly documented for several species of mammals and for some invertebrates (see reviews by Kimeldorf and Hunt, 1965; Smith, 1971).

Intensive behavioral and electrophysiological studies have revealed that detection is mediated primarily *via* visual and olfactory sensory systems in mammals. Little is known about the routes of detection available to lower organisms. Prompt behavioral reactions to x-rays by coelenterates (Kimeldorf and Fortner, 1971) indicates that a highly organized receptor system is not essential for detection. Periodic attempts to identify the mechanisms by which ionizing radiation leads to prompt, reflex-like responses of arthropods have implicated photoreceptors (Axenfeld, 1897; Baylor and Smith, 1958; Smith and Kimeldorf, 1964; Terwilliger and Levy, 1964). Other sensory systems have not been studied, yet arthropod antennae are laden with a variety of sensory receptors and, as distinct appendages, are well suited to manipulative procedures and electrical recording.

The primary objective of the present study was to seek out and characterize prompt behavioral responses given by insects to bursts of ionizing radiation, and to uncover the mechanisms through which detection occurs. In this manner, we hoped to extend the body of knowledge on a topic that has developed almost entirely from studies on selected vertebrate species. Carpenter ants were chosen as subjects for study because some species have been reported to respond immediately (within four seconds) to x-rays (Hug, 1960), yet nothing is known about how radiation effects these responses in ants.

### METHODS

#### *Behavioral studies*

Major worker ants (10-15 mm long) of the species *Camponotus herculeanus* served as experimental subjects for this study. Ants were collected from nests within rotting logs near Corvallis, Oregon. The ants were maintained in the laboratory at room temperature and an approximate 12/12 photoperiod, for at

<sup>1</sup> This investigation was supported by the General Research Fund, Oregon State University Graduate School, and by USPHS, Food and Drug Administration, Bureau of Radiological Health under research grant RL 00119. D. L. Martinsen was the recipient of a NSF Faculty Fellowship and a traineeship in environmental toxicology, USPHS grant ES 00055, during this study.

<sup>2</sup> Present address: Department of Biology, Linfield College, McMinnville, Oregon 97128.

least several days prior to experimentation. Water was provided with moistened cotton pads, and food consisted of bits of insect bodies and grains of sugar.

Prompt, reflex-like responses of ants to x-rays are defined as reactions that were elicited within a few seconds of the onset of exposure and were transient, ceasing either during or soon after termination of exposure. Some consistent, readily-identifiable prompt responses included head bobbing, brisk waving of the antennae, and a startle response involving an abrupt, rapid running behavior. The antennal reaction and the running behavior were selected for quantitative study. Latency of response was defined as the time duration from the onset of exposure to the start of a specific response. Strength or intensity of the running response was assumed to be reflected by the speed of running and was determined by measuring the distance traveled per unit time (cm/sec or cm/min). Other characteristics of prompt responses that were expressed quantitatively were the per cent subjects responding, and the duration of a response.

X-ray stimuli were delivered with a General Electric Maxitron 300 therapy unit. The port of the x-ray unit was positioned above an ant exposure chamber, which in turn rested atop 15 cm of high-density rubber pads in order to maximize backscatter. Most exposures were made at 300 kVp and 20 mA, half value layer equivalence (HVL) = 1.8 mm aluminum (Al). Exposure rates below 0.7 R/sec were achieved by either increasing the beam filtration with thin absorbers, or decreasing the maximum kilovoltage applied to the tube. These variations in beam quality were employed only to determine the exposure rates below which no prompt responses could be elicited. To measure an exposure rate, a Victoreen thimble was inserted into the center of an ant test chamber and exposed under experimental conditions. The distance between the x-ray target and the chamber was then adjusted to yield the desired exposure rate. The test chambers for ants were designed to be small enough, relative to the exposure field, that the measured variation in rate throughout the chamber was usually not greater than the variation among three readings taken at the center of the chamber.

Ants were exposed in single-compartment, 30-cc plastic tissue culture flasks (2 cm × 3.5 cm × 8 cm). The chambers were perforated to provide ventilation and easy introduction of food and water. Experimental subjects were transferred from the main laboratory colony to the chambers between 24 and 30 hours prior to exposure.

Remote observation of ants during x-ray exposure was made by a closed-circuit television system. A television camera, equipped with an f 1.4, 25 mm lens, was positioned close to the exposure chamber in the x-ray room. A TV monitor and video-tape deck were on line and set up in the x-ray control room. An audiochannel of the tape deck allowed the recording of a tone emitted by the x-ray generator that corresponded to the precise period of exposure. Tapes were replayed in the laboratory for the extraction of data on the latency, strength, etc. of the behavioral response in question.

Preliminary tests revealed no difference in the responses between ants exposed individually and those exposed in small groups. Thus, unless stated otherwise, ants were maintained and subjected to test stimuli in groups of five to eight ants per chamber. To minimize the risk that spontaneous changes in behavior would be interpreted as radiation-induced, subjects were not exposed

until they achieved and maintained a resting posture for at least one minute prior to exposure.

Several odors were used to test the behavioral responses of normal and experimental subjects to olfactory stimulation. The strongest and most predictable response (immediate attack behavior) was elicited by the natural odor of a foreign ant (an ant of a different species). This stimulus was presented by introducing a freshly-killed foreign ant, impaled on a toothpick, into the test subjects' chamber. The odors of nestmates were introduced as controls, to insure that visual or mechanical cues were not involved in the responses observed.

Both partial and complete antennectomy and ophthalmectomy were employed to assess the role of antennal and visual receptors in the detection of ionizing radiation. Operations were performed on ants anesthetized with carbon dioxide. Control subjects for each experiment underwent a sham operation procedure. After perfection of surgical technique, the mortality of experimental subjects was no greater than that of anesthetized controls (0–20%). In all cases, a post-operative recovery period of at least 24 hours was allowed prior to experimentation. One or both antennae (or parts thereof) were removed by cutting between two adjoining segments with iridectomy scissors. Care was taken to cut precisely at the intersegmental joint, thereby preventing excessive loss of body fluid, and facilitating quick recovery. To insulate the intact antenna from odors in one study, purified white shellac was applied to the antennal flagella with the aid of a fine-tipped brush. The shellac was nontoxic, dried quickly, and formed a seamless coat over the flagella. For ophthalmectomy, a fine watchmakers forcep was employed to remove the cuticle, corneal lens, and underlying crystalline structures containing the receptor cells. It was not entirely possible to be certain by visual inspection through a microscope that all photoreceptive cells in the compound eye had been removed or destroyed, but operated subjects did not, after dark adaptation, respond to bright flashes of light, as did sham-operated controls.

### *Electrophysiological studies*

*The Electroretinographic response (ERG).* Preparation of the eye for ERG recording involved severing the head from the body, removing the antennae and mouthparts, then partially imbedding the isolated head in wax. The wax mount was held in a stereotaxic device. With the aid of micromanipulators and a binocular microscope, a platinum-clad microelectrode was inserted into the cuticle of the eye to a depth of about 0.2 mm, and a platinum reference electrode was positioned in the base of the head. All plastic components were painted flat black, to prevent any potential radiation-induced fluorescence from stimulating the eye.

Repeated tests showed that the responsiveness of the eye did not begin to decrease for at least three hours after initial records. All results were obtained from fresh preparations. The ERG signal was amplified and recorded on a Grass VII oscillograph in conjunction with a Grass 7PIA low-level DC pre-amplifier.

We exposed the eyes of ants to stimuli of light, beta radiation, and x-rays.

The typical procedure was as follows: Each eye was dark-adapted for several minutes, then stimulated by flashes of light for standardization of response, followed by test stimulation with ionizing radiations. The physiological integrity of the preparation was tested at intervals by checking the response to visible light stimuli. In this way, comparisons could be made between responses given to ionizing and non-ionizing radiation, using each eye as its own control.

For stimulation by light, a Bausch and Lomb diffraction grating monochromator in series with a 150-watt xenon lamp system was used to provide 500 nm wavelength radiation. The light beam was passed through the aperture of a light-tight box containing the eye preparation. Flash duration was controlled by a leaf-type camera shutter fixed to the exit slit of the monochromator. Bimetallic quartz-bonded interference filters of neutral density were used to change the dose rate over several log units. Intensities were measured in ergs/cm<sup>2</sup>-sec at the level of the eye with a YSI-Kettering radiometer.

Beta radiations were provided by compact, sealed applicators (Tracerlab) containing either 100 mCi or 50 mCi of strontium-90 in equilibrium with yttrium-90. The maximum energy of beta radiation emitted by the source was 2.25 MeV. Dose rate measurements were made by means of lithium fluoride thermoluminescent dosimetry. Very small calibrated lithium fluoride dosimeters were positioned at the site of the eye, and the absorbed dose determined for timed exposures in the range of 1/20 to 1/60 seconds. For presentation of the eye to beta radiation, an apparatus described by Smith and Kimeldorf (1964) was employed. The outer margin of a steel disc was positioned between the beta source and the eye preparation. The disc had two holes drilled in its margin, 180 degrees apart, and was aligned so that rotation of the motor-driven disc exposed the eye preparation to two flashes of radiation per revolution. As one hole exposed the eye to beta radiation, the opposite hole exposed a photocell to a flash of light from a shielded light source. Output from this photocell led into one channel of the oscillograph and served as a stimulus monitor.

X-ray exposures were made with a diagnostic x-ray unit, operated at 70 kVp with tube currents between one and 10 mA, and a measured HVL of 0.58 mm Al. The x-ray tube head was mounted on top of a lead-lined cabinet, which served as the exposure chamber. X-ray exposures were controlled by a solenoid driven focal plane shutter constructed of lead. Limitations imposed by the relatively high-inertia shutter made it impossible to attain stimulus rates of greater than four per second or durations of less than  $\frac{1}{10}$  second. The onset and duration of each x-ray exposure was monitored by a photocell that was covered with a fluorescent screen and enclosed in a light-proof capsule. The photocell was suspended in the exposure chamber directly beneath the eye preparation and its output was fed into one channel of the recording oscillograph. Exposure rates were varied by changing tube current. A low energy 25 R thimble chamber was positioned at the site where the eye was to be exposed and measurements were made at several tube current settings. The exposure rate over the range tested was linearly related to tube current and ranged from 0.3 to 2.7 R/sec.

*The Electroantennographic response (EAG).* The composite bioelectric response of the insect antenna, named the electroantennogram by Schneider (1957a), has been less well studied than the ERG. When an adequate olfactory stimulus

impinges on the insect antenna, a relatively slow electrical potential difference develops between the tip and the base. This potential is thought to reflect the summed receptor potentials of many olfactory sensory neurons (Schneider, 1963).

The ant antenna was isolated from the rest of the body to record the EAG, because stimuli often evoked potentials associated with muscular movements of the mandibles or of the scapus muscles joining the antenna to the head. The isolated antenna was mounted on a cork platform. Two platinum electrodes were affixed to separate micromanipulators. The tip of one electrode was then inserted into the base of the antennal scape. The other electrode was secured within a fine-tipped glass pipette filled with 1.0% saline, into which the distal segment of the flagellum was manipulated. Amplification and recording was accomplished by the systems described for ERG measurements. All results were obtained within 30 minutes of isolating an antenna, after which dehydration usually resulted in diminished responsiveness.

To administer odor stimuli, an air-jet system was devised which provided a constant flow of air over the isolated antenna, into which a test odor could be injected periodically. Odor stimuli were presented by dipping the tip of a clean glass rod into a test solution and passing it back and forth through the air stream impinging on the antenna. Control tests were made in the same way, using distilled water rather than an odorous solution. The approximate onset and duration of each stimulus was recorded on a separate channel of the oscillograph using a hand-operated switch.

The 50 mCi radioactive source was used to provide beta radiations for the antenna study. The air-jet was removed and a lead shield was placed over the antenna, with a small window located directly above and one centimeter out from the antenna. To deliver a train of stimuli, the beta source was passed back and forth over the window. The rate of movement of the source determined the stimulus duration, whereas intensity was varied by altering the distance between the source and the antenna. Dose rates from 0.15 to 3.2 rads/sec were available by this procedure. Each exposure to beta radiation was preceded and followed by a sham exposure with a Tracer-lab non-radioactive applicator. Sham exposures were made to detect any potential artifacts that could be induced by the manipulation of the radiation source close to the antenna.

## RESULTS

### *Prompt behavioral responses to x-rays*

*Intact (normal) subjects.* Below an exposure rate of 0.05 R/sec, no ant gave any behavioral evidence of detection. Above this threshold rate, the proportion of subjects responding by vigorous movements of antennae was a direct function of exposure rate (see tabular data, Figure 1). This response occurred through a wide range of exposure rates and beam qualities. It appeared to represent a taxis or orientation response. Antennal waving was always the first response given to x-rays, and often was the only response at low exposure rates (less than 0.5 R/sec). The latency of the antennal-waving response was an inverse function of the exposure rate (Fig. 1). Note that the latency varied through three orders of magnitude. Lower exposure rates could be achieved only by using different beam qualities.

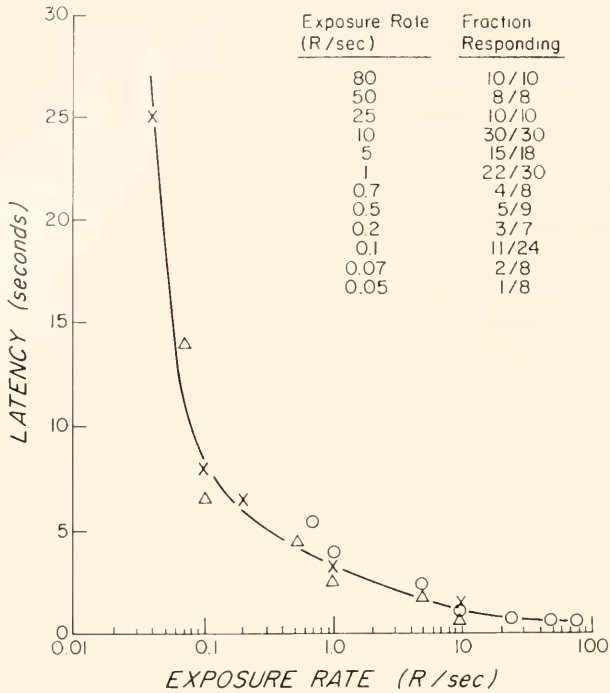


FIGURE 1. Average latency of the antennal-waving response (graph), and fraction responding (tabular data), as a function of exposure rate. Different symbols represent different beam qualities:  $\circ$  = 300 kV, 20 mA, 1.8 mm Al. HVL;  $\times$  = 300 kV, 20 mA, 4.0 mm Cu. HVL;  $\Delta$  = 100 kV, 20 mA, 0.25 mm Al. HVL.

Since there was no significant difference in the average latency of the response at points of common exposure rates (10, 1 and 0.1 R/sec), the curve in Figure 1 was fitted to an average value at each exposure rate where more than one beam quality was employed.

Another behavior that consistently appeared as a prompt response was a burst of rapid running activity along the floor and all sides of the exposure chamber. As with the antennal response, the incidence varied directly, while latency varied inversely, with exposure rate. The exposure rate threshold for the running response appeared higher than for the antennal response since it was not observed with less than 0.2 R/sec. Rates of 1 R/sec or less often evoked only the antennal response; when running did occur it was typically several seconds later in appearance than the antennal-waving response. At 10 R/sec and above, the running response began simultaneously with the antennal response, and both responses were exhibited by 100% of the subjects. The intensity of the running response appeared variable until it was analyzed as a function of exposure rate. When speed was taken as a measure of the strength of the running response, it was found to be related to the rate of x-ray exposure (Fig. 2). Speed of running appeared to be independent of exposure rate between 0.5 and 5 R/sec, then increased dramatically up to 80 R/sec.

Some ants exhibited adaptation during exposure at rates below 0.5 R/sec during prolonged exposures, *i.e.*, the antennal response waned, and subjects ceased to give behavioral evidence of detection while the beam was still on. Rates of 1 R/sec or higher, however, usually caused antennal waving and running to persist throughout a 30 second exposure period. During long (minute) exposures, ants would periodically stop running and clean their antennae, but these brief pauses were transient. Rapid running was quickly resumed, and there were no clear indications of adaptation or habituation at rates of 1 R/sec or higher.

On termination of exposure, most subjects exhibited an abrupt cessation of running, accompanied by vigorous antennal-cleaning behavior. Thus, both the onset and the cessation of irradiation elicited detectable changes in the behavior of ants. At rates of 10 R/sec or higher, the "off" response was transient, lasting

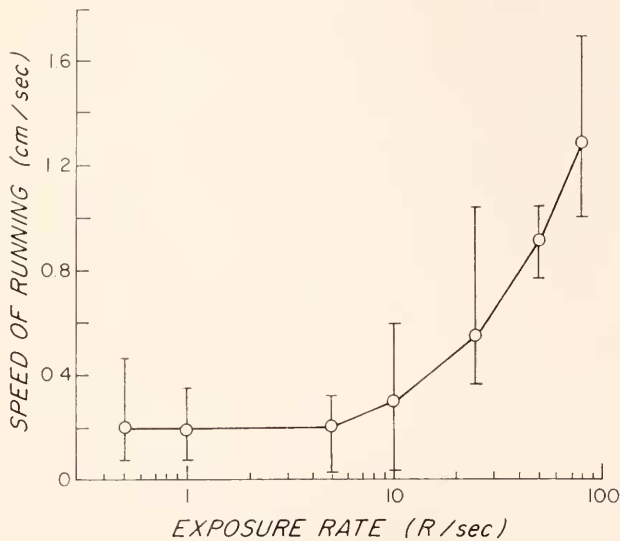


FIGURE 2. Average intensity of the running response as a function of exposure rate. Each point is an average of 5-8 subjects. Vertical bars show range of response.

only a few seconds. A high level of activity then reappeared, even though the beam had gone off.

A test was made to determine the effects of repetitive exposure on prompt responses. Alterations in the response pattern might be expected as a result of sensitization, fatigue, or direct tissue damage. A group of 10 ants was subjected to six exposures during a two-day period. Each exposure, at 80 R/sec, lasted for one minute. There was no measurable change in the latency or the strength of prompt responses with successive exposures.

*Ophthalmectomized subjects.* The prompt nature of the foregoing responses indicated that the nervous system was being stimulated, either directly or indirectly, possibly through specific sensory pathways. To test for potential involvement of the visual system, we bilaterally ophthalmectomized two groups of ants ( $n = 5/$

group) and exposed them to 10 R/sec for 30 sec. Responses were just as strong as those given by normal controls. The average latency of the running response was somewhat longer in eyeless subjects (1.2 sec) than in normal ants (0.9 sec), but this difference was not statistically significant. Thus, the visual system was not primarily responsible for the behavioral reactions given by ants to brief exposures of x-rays.

*Antennectomized subjects.* Several experiments were performed in an attempt to elucidate the role of the antennae in responses given by ants on exposure to x-rays. When stimulated at 10 R/sec or higher, antennal-waving and running behavior began simultaneously, usually within one second of the onset of exposure. To determine whether intact antennae were essential for the running response to be elicited, we removed both antennae from each of six subjects, then exposed them to 10 R/sec for 30 seconds. None of the antennectomized ants gave any detectable response during exposure. Antenna thus appeared essential for the detection of a stimulus which led to the running response given by normal subjects.

A subsequent experiment established that ants missing only the flagellar portion of both antennae were not capable of detection. In the type of antenna possessed by ants, aggregations of specialized sensory nerve endings are found only in the flagella. By contrast, antennal muscles and motor components of the large antennary nerves are restricted to the scapes (Schneider, 1964). The sensory component of the nerve tract extends from the deutocerebrum through the scapes into the flagella. Since ants with scapes intact did not respond to x-rays, ionizing radiation must act primarily or exclusively on the receptor neurons distributed along the segments of the antennal flagella.

Thus, a study was made to determine which of the 11 flagellar segments were involved in detection. Eleven experimental ( $n = 10/\text{group}$ ) and one control groups were prepared. Members of each experimental group had "n" segments removed from both antennal flagella. The "n" was equal to one segment for the first group, and was increased by one for each succeeding group, such that ants in group 11 had all eleven segments (the entire flagellum) missing from both antennae. Each ant was maintained individually and exposed to 10 R/sec for 30 seconds. Latency and strength of behavioral responses were scored, as well as the per cent responding to exposure.

The latency of the running response is plotted in Figure 3 as a function of the number of segments removed. Per cent subjects responding is also tabulated in this figure. Note that nearly all ants in each group missing fewer than six segments responded as quickly as did the controls. Most ants missing 6, 7, or 8 segments did respond, but the average latency of the response increased markedly. Almost none of the subjects missing nine or more segments gave evidence of detection during the exposure period (in other words, latency to response became infinite).

The strength of prompt responses (frequency of antennal-waving and speed of running) followed the same pattern as latency. Ants with more than six segments intact responded as strongly as did the controls. When the sixth, seventh, and especially the eighth segments were missing, subjects usually signalled detection only by mild waving of the antennal "stubs," and responded with distinctly less intense running activity.



These results showed that carpenter ants deprived of sensory input from certain of the distal segments of the antennal flagella either did not detect a stimulus, or the stimulus was not sufficiently strong to cause behavioral responses typically given by normal subjects on exposure to x-rays.

The next question concerned the specific kinds of receptors involved. Antennae of ants contain sensory receptors specialized for olfaction, mechanoreception, hygroreception, and thermoreception. While receptors for most senses are also found elsewhere on the body, behavioral studies have shown that, for some hymenopterans, antennal flagella are the exclusive site of olfactory receptors (Schneider, 1964). To test the hypothesis that olfactory receptors were responsible for detection of x-rays by ants, 12 groups of ants were prepared for tests with various odors ( $n = 3-5$ ). As in the previous experiment, members of each group had "n" segments removed from both flagella, where  $n = 0$  for the intact controls and was increased by one for each succeeding group. Ants were individually presented with the odor of a foreign ant, and then the odor of a nestmate. Results were striking: most ants missing up to nine segments reacted aggressively toward the foreign odor, attacking or attempting to escape from the source. These subjects accepted or ignored the odor of a member from the same nest. In sharp contrast, ants with nine or more flagellar segments missing were not capable of discriminating between the odors of "friend" and "foe." Thus, ants with up to nine segments removed from their antennal flagella responded to x-rays (Fig. 3) and were also capable of odor dis-

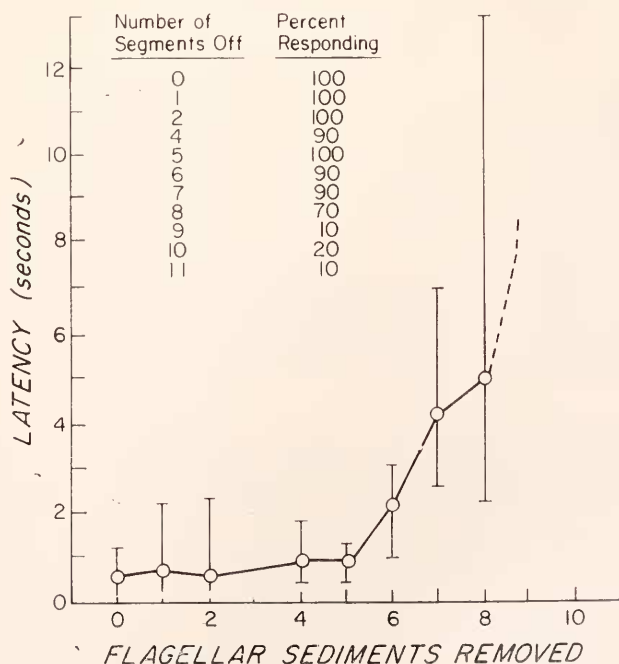


FIGURE 3. Latency of the running response as a function of the number of segments amputated from both antennal flagella. Vertical bars show range ( $n = 10/\text{group}$ ). Exposure rate, 10 R/sec. Per cent subjects responding is given in tabular form.

crimination. Those with nine or more segments amputated did not respond to 10 R/sec and could not make the odor discrimination. These results suggest that olfactory receptors participate in the detection of x-rays which result in prompt behavioral reactions of carpenter ants.

Ionizing radiation might have constituted an effective stimulus for flagellar receptors, or acted indirectly via the production of radiolytic byproducts in air which, in turn, stimulated the receptors. An impervious coating of shellac on the antenna should inhibit the potential action of ozone and yet be easily penetrated by x-rays. Accordingly, the antennae of each of 10 ants were shellacked, after which ants were tested for and found incapable of discriminating between odors. Ants were then individually exposed to 10 R/sec for 30 seconds. Seventy per cent of

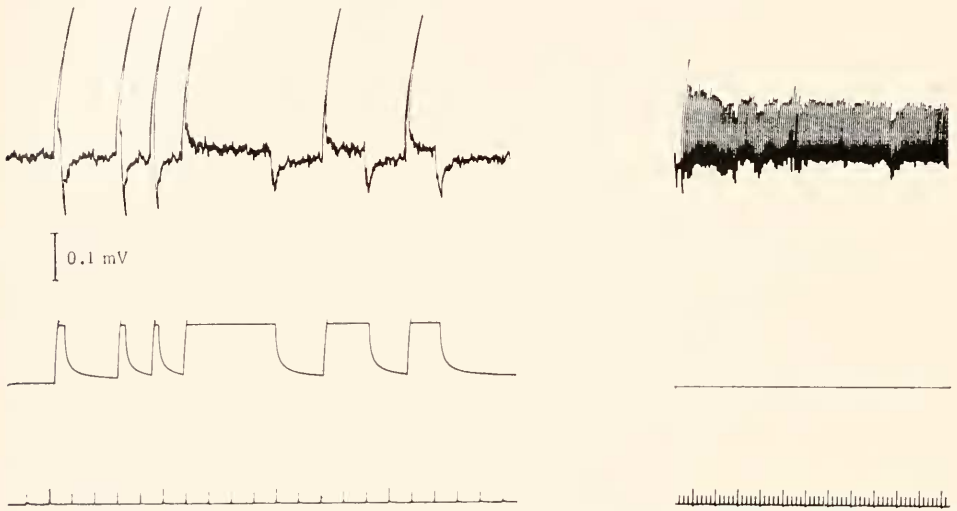


FIGURE 4. ERG response to light (500 nm) stimuli ( $5.78 \times 10^8$  ergs/cm<sup>2</sup>/sec) of varying duration (left) and with repeated exposures (40 ms duration) at 2.5 bursts/sec. Top tracing is the ERG response; middle tracing, stimulus monitor; bottom tracing, the time record in seconds. The stimulus marker was inoperative in the right record.

the experimental subjects responded by running behavior within five seconds, but the average latency of response, 1.7 seconds, was twice that of normal subjects. Speed of running was highly variable, and generally weaker than that of controls. These results suggest that behavioral responses were due, at least in part, to x-rays acting in or on the immediate surface of the antennal receptors.

While the responses of light-adapted ants appeared to be mediated entirely by antennal receptors, it seemed possible that dark-adapted ants might respond to x-rays through visual stimulation. To test this hypothesis, 10 antennectomized ants were exposed to 10 R/sec for 20 seconds after the lights in the x-ray room had been dimmed such that the outlines of subjects were barely visible on the TV monitor. No behavioral responses were observed to occur in any subject.

*Bioelectric responses of sensory receptors*

*The isolated eye.* The results of behavioral studies illustrated that a visual route of detection need not be postulated to explain prompt behavioral reactions to x-rays. However, the isolated dark-adapted ant eye was found to detect stimuli of ionizing, as well as non-ionizing radiation. Electroretinograms were obtained with light, x-ray, and beta-ray stimuli. Results of the light-induced ERG will be presented first, to provide a base against which responses of the eye to ionizing radiations can be compared.

A rapid increase and decrease in light intensity evoked correspondingly rapid changes in the electrical potential within the eye that are reflected in the ERG (Fig. 4). A slow increase in light intensity did not elicit a response in the ERG, even though the same absolute change in intensity occurred in both cases. With an effective stimulus, regardless of duration, the "on" response (upward deflection)

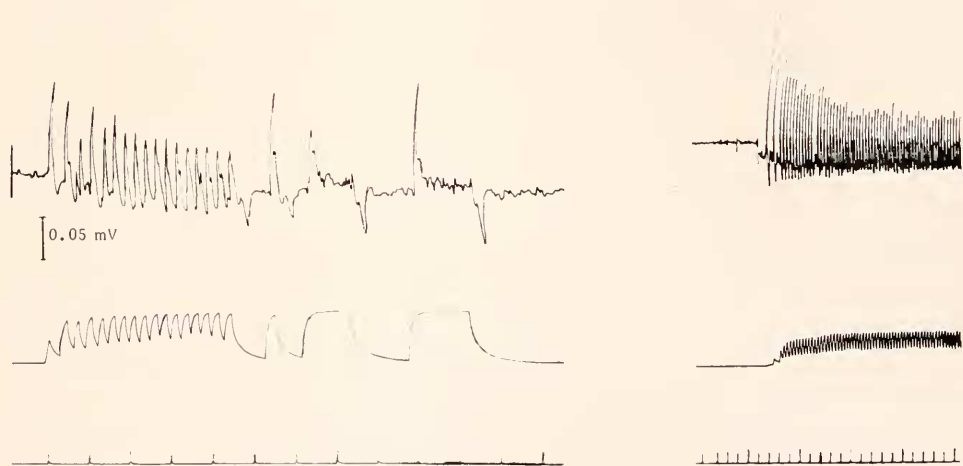


FIGURE 5. ERG responses to a train of x-ray flashes of varying durations (left) and the decrease in response with reduction of interstimulus interval. Tracings as in Figure 4. In right record, flash rate and duration were 3.5/sec and about 1/10 sec respectively. Exposure per flash was 0.22 R.

always fell rapidly to the baseline, and cessation of stimulation yielded a deflection of opposite polarity ("off" response). There was a decrease in amplitude of successive responses when the interstimulus interval was less than the complete recovery time of the dark-adapted eye. Also, the strength (amplitude) of the light-induced ERG was a direct function of stimulus intensity.

All eyes exposed to brief x-ray stimuli gave measurable ERGs that were very similar to those elicited by light. Rapid increases and decreases in intensity resulted in sharp "on" and "off" responses, whereas a slowly-changing intensity was ineffective in causing a response. A series of responses to stimuli of various durations are seen in the left part of Figure 5. This record shows that, as with visible light stimuli, x-ray stimulation could modify the responsiveness of the eye to subsequent stimuli. If the interval between exposures was very short, the eye re-

sponded at successively reduced strength. Full recovery occurred within one second after termination of a train of rapid stimuli, as manifest by a subsequent response of maximum amplitude (middle of left record, Fig. 5). The right record in Figure 5 shows that the decrease in response with repeated stimulation leveled off within several seconds, after which the eye continued to respond indefinitely, at reduced strength.

The strength of the ERG response was a direct function of the strength of the stimulus, for both x-rays and light. Amplitude-intensity curves for both stimuli exhibit similar slopes through the entire range of intensities used in this study. This and other similarities of x-ray and light-induced ERGs suggests that similar mechanisms might underly the ERG response in the case of both stimuli.

The threshold exposure rate of x-rays to elicit a bioelectric response from the eye was below 300 mR/sec, the lowest exposure rate used in this study. At this rate

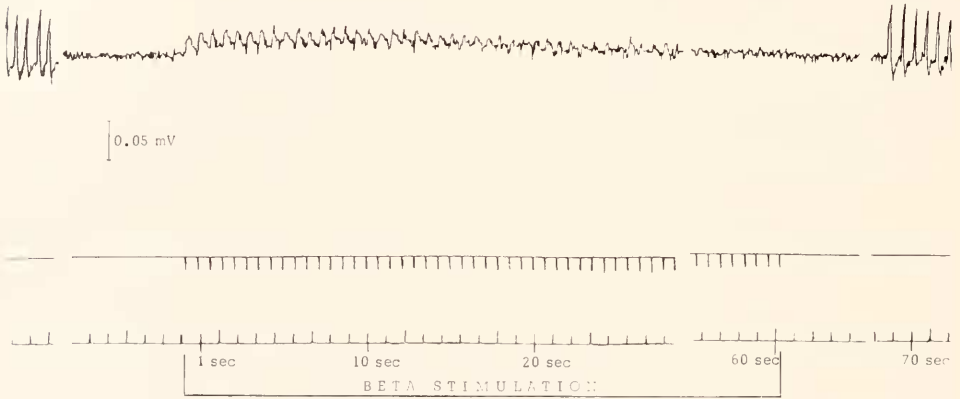


FIGURE 6. ERG responses to beta radiation, preceded and followed by responses to light. As indicated by the interruptions in tracings, sections of the original record were deleted in order to display the series on one line. Beta stimulus conditions were 7.7 mrad per 1/30 sec flash and 1.6 flashes per second. Traces are as in previous records.

almost all eyes gave small but distinct peak responses, even when the flash duration was only 100 milliseconds, resulting in an exposure per flash of 30 mR.

Only five of nine eyes tested responded to beta radiation with measurable ERGs. Typical responses to a series of beta stimuli (7.7 mrad/flash) are illustrated in Figure 6. Note that, although the upward deflection of each "on" response rose promptly with the onset of each stimulus, it decayed slowly back to the baseline and there was not a conspicuous "off" response. There was a decrease in the amplitude of responses to succeeding beta stimuli, but this decrease differed from that observed on repeated stimulation with light or x-rays in three ways. (1) The decrease continued until the response peaks merged imperceptibly with the noise level of the amplifier and recorder (Fig. 6). (2) Once the eye ceased to give measurable responses to beta stimuli, no further responses to beta stimuli could be elicited, even after periods up to one hour of rest. An obvious

assumption was that beta radiation was damaging the eye, but repeated tests showed that such an eye always responded strongly to light stimuli (compare the initial and final light-induced ERGs in Figure 6). Thus beta stimulation, while rendering the eye measurably insensitive to subsequent beta stimuli, did not inactivate the photoreceptive mechanism that was responsive to light. (3) The duration of the interstimulus interval ("rest" period) had no effect on the decrease in response amplitude to successive beta stimuli. If a train of beta stimuli was termi-

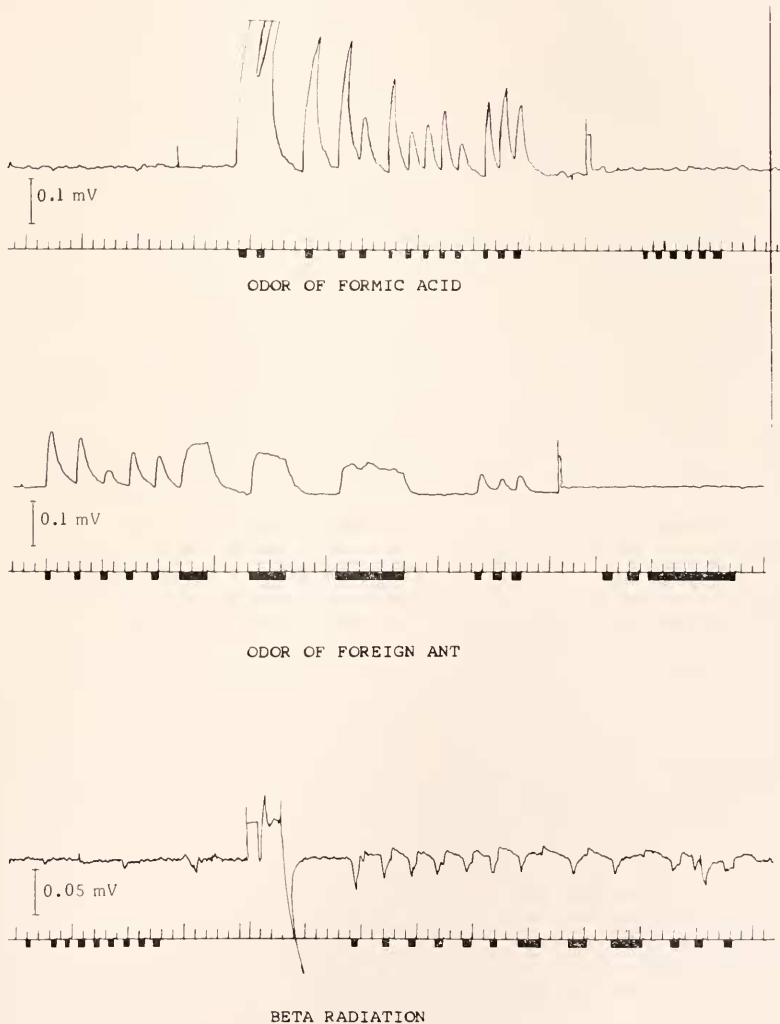


FIGURE 7. Representative EAGs from one isolated antenna exposed to three different stimuli. For each record, upper trace is the EAG, lower trace is the time in seconds. Solid blocks beneath time marks represent the onset and duration of stimuli. Sham tests follow or, in the bottom record precede, the true stimulus. Deflections between exposures and sham tests are artifacts related to operation of the amplifier and recorder.

nated prior to complete loss of responsiveness and then resumed several minutes later, the decline in amplitude progressed as if the two trains had been delivered uninterrupted. Thus, the loss of response to beta stimuli seemed irreversible, and appeared to depend on total absorbed dose. These findings indicate that the mechanisms of action on the eye for beta particles may differ from that for photons in visible and x-ray range.

The threshold dose rate required to elicit an ERG was approximately 62 mrads/sec. At this dose rate, a train of stimuli from the 50 mCi source elicited small but distinct response peaks from one of the eyes studied when the flash duration was 1/20 sec (3.1 mrads/flash).

In spite of the unexplainable differences between x-ray and beta-induced electroretinographic responses, these studies clearly established that, under conditions of dark-adaptation, the eyes of ants detected the presence of high-energy photon and electron stimuli. Such detection, however, apparently did not initiate the prompt behavioral reaction of ants to x-rays.

*The isolated antenna.* Electroantennograms were obtained from several isolated antennae on exposure to the odor of a foreign ant, the odor of formic acid, and to beta radiation.

Six of eight antennae responded to the odor of formic acid, which is an excitatory pheromone elaborated by ants under natural conditions. The large, upward deflections in the top record in Figure 7 correspond to 400–800 millisecond exposures to this odor. This region of the figure is followed by an illustration that exposure tests to the "odor" of distilled water did not result in responses. An upward deflection means that the tip of the antenna became negative relative to the base. EAGs elicited by the odor of a foreign ant are shown in the middle record of Figure 7. Sham tests on the latter half of the record were again negative. The odor-induced EAGs were monophasic and decayed to the baseline relatively slowly, regardless of stimulus duration.

Four of eight isolated antennae gave an EAG to beta stimuli. The bottom record in Figure 7 shows typical responses to a train of beta flashes of various durations, delivered at a dose rate of approximately 2.2 rads/sec. When compared to odor-induced EAGs, beta responses were of opposite polarity, and response peaks were somewhat sharper, with the decay of each deflection to the baseline beginning immediately, irrespective of stimulus duration. There also appeared to be a slight "off" response of opposite polarity upon cessation of some of the beta stimuli.

The amplitude of response was noted to vary directly with dose rate. Small EAGs to beta stimuli were elicited by 380 mrads/sec, whereas 150 mrads/sec failed to elicit measurable responses, regardless of the duration of exposure. Thus, the threshold dose rate range required to elicit an EAG was between 150 and 380 mrads/sec.

These data offer at least qualitative support for the evidence derived from studies of behavior that sensory receptors on the antennal flagellum can detect a stimulus that could lead to the prompt behavioral reactions of carpenter ants to ionizing radiation.

## DISCUSSION

The principal objective of this study was to seek out and characterize behavioral responses given by carpenter ants to bursts of ionizing radiation and to uncover the potential mechanisms through which detection could occur. In this manner, we hoped to extend the body of knowledge on a topic that has been developed almost entirely from intensive studies on selected vertebrate species.

Prompt behavioral responses were found to occur at exposure rates as low as 0.05 R/sec. Characteristics of each response, such as latency, strength, duration, and per cent subjects responding, were strongly exposure-rate dependent. This suggested that excitable tissues were stimulated by something inherent in or accompanying exposure to x-rays.

Any artifactual cue would have had to vary in intensity with the exposure rate. Possible candidates included noise from the x-ray machine and radiation-induced fluorescence in the walls of the exposure chambers. With respect to noise cues, subjects did not respond when the chamber was shielded from x-rays but not from sound. With respect to fluorescence, exposures were made in a well-lighted room in order to mask any potential effects of fluorescence. Another possible stimulus was atmospheric by-products of ionization, such as ozone or oxides of nitrogen. Little work has been done on this problem, since it is difficult to measure the concentration and duration of radiolytic by-products resulting from irradiation of air. However, ants with shellacked antennae did exhibit detection on exposure, suggesting that the effect was mediated within or on the immediate surface of antennal receptors. Thus, none of these concomitants of exposure appeared to be responsible for behavioral reactions to x-ray stimuli.

Previous attempts to identify the physiological mechanisms by which x-ray stimuli elicit behavioral responses from arthropods have implicated photoreceptors. Terwilliger and Levy (1964) discovered that crabs (*Uca*) ceased to give an "off" response when their eyes had been extirpated. Another series of investigations (Smith, Kimeldorf and Hunt 1963; Smith and Kimeldorf, 1964) revealed that various species of moths gave prompt motor responses to ionizing radiation if they were exposed in a dark-adapted state; in a well-illuminated room no responses were observed to occur. These investigators used behavioral and electrophysiological techniques to show that the motor responses of moths were mediated by the dark-adapted visual system, and occurred at exposure rates as low as 10 mR/sec. In contrast to moths, carpenter ants gave motor responses when exposed to x-rays (50 mR/sec) during the light-adapted state. Moreover, bilateral ophthalmectomy did not prevent the initial responses in ants.

Although behavioral responses occurred in the absence of visual detection, the isolated ant eye gave a strong reversible ERG to x-rays. The characteristics of the resulting electroretinogram were very similar to those elicited by light stimuli, suggesting similar mechanisms of action of light and x-rays on the photosensitive pigment molecules in the ommatidia. ERG responses were also evoked by beta radiation at dose rates as low as 62 mrad/sec, with a flash duration of 1/20 sec. Thus, a threshold absorbed dose per flash of only 3.1 mrad was an effective stimulus for the dark-adapted eye. This is somewhat higher than the 0.25 mR exposure required to elicit an ERG response from the eye of the moth, *Pseudaletia unipuncta*, reported by Smith and Kimeldorf (1964). The irreversible decline in

responsiveness of the ant eye to a series of beta stimuli has been observed in the eyes of other arthropods (Jordan and Kimeldorf, 1971), but is not understood. Beta-induced ERGs appeared to involve a different or an additional mode of action than that underlying the responses to light and to x-rays.

The occurrence of behavioral responses in light-adapted carpenter ants and in ophthalmectomized subjects indicated the presence of a more relevant detector system. Results of the series of experiments on antennectomized ants leave little doubt that, under the conditions of this study, the principle route by which x-ray stimuli evoked immediate, transient behavior changes in carpenter ants was via receptors (probably olfactory receptors) in some or all of the nine distal segments of the antennal flagella.

Electroantennograms were elicited from isolated antenna by several olfactory stimuli and by ionizing radiation. An understanding of the differences between odor-induced EAGs and those given upon exposure to pulses of radiation must await further study. Detrich Schneider, the worker who has studied extensively the EAG response, reported that the form of the EAG depends on several unknown factors. Different odors, and even the same odor at different times, have resulted in markedly different electroantennograms in silkworm moths, *Bombyx* (Schneider, 1957b, 1962).

The threshold dose rate to elicit an EAG from half of the antennae studied was between 150 and 380 mrad/sec of beta radiations. Within this exposure range (at 200 mR/sec) about half of the ants also gave behavioral evidence of x-ray detection (Figure 1), but the response time was considerably longer for the antennal-waving response than for the appearance of an EAG. Strict quantitative correlations between behavioral and electrophysiological responses are not possible, since different kinds of ionizing radiation were employed as stimuli. Also, the precise relationship between an electrical response and the motor system involved in the behavior is not known as yet.

No previous study has implicated olfactory (or any antennal) receptors in the prompt detection of ionizing radiation by arthropods. As mentioned earlier, olfactory receptors are known to participate in the prompt detection of x-rays by mammals like rats and monkeys (Smith, 1971). Threshold exposure rates for bioelectric responses from the olfactory bulbs of rats (Cooper, 1968), and for behavioral evidence of detection in rats (Garcia, Buchwald, Feder, Koelling and Tedrow, 1964) and in monkeys (Smith and Tucker, 1969) range from three to 50 mR/sec. These rates are lower than the threshold exposure rates found necessary to elicit comparable responses in ants (above), but the important point is that the olfactory system now appears to be intimately involved in prompt responses to ionizing radiation in animals as diverse as ants and primates.

#### SUMMARY

Carpenter ants exhibited behavioral responses to 10 R/sec (x-rays) within one second of the onset of exposure. Within the range of 0.05 R/sec to 80 R/sec the strength and duration of responses were proportional to the exposure rate. Latency was inversely related to exposure rate.

A comparison of behavioral reactions of normal subjects with subjects whose eyes or antennae had been removed or shielded, revealed that sensory receptors



(probably olfactory) on the antennal flagella were primarily responsible for detection and led to the onset of prompt responses.

Bioelectric potentials were recorded from isolated eyes (electroretinograms) and isolated antennae (electroantennograms) during brief exposures to ionizing radiations. The results largely corroborated evidence derived from behavioral studies that eyes and antennal receptors are sensitive detectors of ionizing radiation. The strength of bioelectric responses varied directly with stimulus strength. Estimates of threshold exposures or doses, delivered in fractions of a second, indicated that the eyes and antennal receptors of the ant can detect x-ray and beta radiations in the millirad dose range.

X-ray and visible-light stimuli elicited electroretinograms with very similar characteristics, suggesting a common mechanism of action on the photoreceptor pigment in the ommatidia. The response to beta radiations displayed some unusual characteristics that cannot be explained at present.

#### LITERATURE CITED

- AXENFELD, D., 1897. Die Rontgen'schen Strahlen dem Insectenaug sichtbar. *Zentralblatt für Physiologie*, **10**: 436-437.
- BAYLOR, E. R., AND F. E. SMITH, 1958. Animal perception of x-rays. *Radiat. Res.*, **8**: 466-474.
- COOPER, G. P., 1968. Receptor origin of the olfactory bulb response to ionizing radiation. *Amer. J. Physiol.*, **215**: 803-806.
- GARCIA, J., N. A. BUCHWALD, B. H. FEDER, R. A. KOELLING AND L. TEDROW, 1964. Sensitivity of the head to x-rays. *Science*, **144**: 1470-1472.
- HUG, O., 1960. Reflex-like responses of lower animals and mammalian organs to ionizing radiation. *Inter. J. Radiat. Biol. Suppl.*, **2**: 217-226.
- JORDAN, N. M., AND D. J. KIMELDORF, 1971. The visual response of the purple shore crab, *Hemigrapsus nudus*, to ionizing and non-ionizing radiations. *Experientia*, **27**: 653-655.
- KIMELDORF, D. J., AND E. L. HUNT, 1965. *Ionizing Radiation: Neural Function and Behavior*. Academic Press, New York, 331 pp.
- KIMELDORF, D. J., AND R. W. FORTNER, 1971. The prompt detection of ionizing radiations by a marine coelenterate. *Radiat. Res.*, **46**: 52-63.
- SCHNEIDER, D., 1957a. Elektrophysiologische Untersuchungen von Chemo- und Mechanorezeptoren der Antenna des Seidenspinners *Bombyx mori* L. *Z. Vergl. Physiol.*, **40**: 8-41.
- SCHNEIDER, D., 1957b. Electrophysiological investigation on the antennal receptors of the silk moth during chemical and mechanical stimulation. *Experientia*, **13**: 89-91.
- SCHNEIDER, D., 1962. Electrophysiological investigation of the olfactory specificity of sexual attracting substances in different species of moths. *J. Insect Physiol.*, **8**: 15-30.
- SCHNEIDER, D., 1963. Electrophysiological investigation of insect olfaction. Pages 85-103 in Y. Zotterman, Ed., *First International Symposium on Olfaction and Taste*. Pergamon Press, Oxford.
- SCHNEIDER, D., 1964. Insect antennae. *Annu. Rev. Entomol.*, **9**: 103-122.
- SMITH, J. C., D. J. KIMELDORF AND E. L. HUNT, 1963. Motor responses of moths to low-intensity x-ray exposure. *Science*, **140**, 805-806.
- SMITH, J. C., AND D. J. KIMELDORF, 1964. The bioelectrical response of the insect eye to beta radiation. *J. Insect Physiol.*, **10**: 839-847.
- SMITH, J. C., AND D. TUCKER, 1969. Olfactory mediation of immediate x-ray detection. Pages 288-298 in C. Pfaffmann, Ed., *Olfaction and Taste, Volume 3*. Rockefeller University Press, New York.
- SMITH, J. C., 1971. Radiation, its detection and its effects on taste preferences. Pages 53-118 in E. Steller and J. M. Sprague, Eds., *Progress in Physiological Psychology, Volume 4*. Academic Press, New York.
- TERWILLIGER, R. C., AND C. K. LEVY, 1964. Behavioral response of the fiddler crab (*Uca pugilator*) to light and ionizing radiation. *Physiologist*, **7**: 270.